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6	10151	internal adj standard	USPAT	2002/10/19 09:52
7	18	(435/7.1.ccls. and (mass adj spectrometry)) and (internal adj standard)	USPAT	2003/12/11 12:18
8	661	(mass adj spectrometry).clm,ab.	USPAT	2002/10/19 10:01
9	39	435/4,7.1.ccls. and ((mass adj spectrometry).clm,ab.)	USPAT	2002/10/19 10:01
10	29	(435/7.1.ccls. and (mass adj spectrometry)) and (internal adj standard)	USPAT	2003/12/11 12:18

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TITLE: Quantitation of endogenous substance P by on-line  
microcolumn liquid chromatography/continuous-flow  
fast-atom bombardment \*\*\*mass\*\*\*  
\*\*\*spectrometry\*\*\*

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AB Cat spinal cord substance P (SP) as detd. by the title method agreed well with that detd. by a std. RIA; e.g. anal. value were 39.0 and 40.7 pmol, resp. The most important part of the title method is the immunol. purifn. of the sample prior to mass spectrometric anal. The spinal cord ext. plus the \*\*\*internal\*\*\* std. [D8-Phe8]SP was applied to an \*\*\*immunoaffinity\*\*\* column contg. rabbit polyclonal antibodies immobilized on CNBr-activated Sepharose 4B and eluted with 20% aq. formic acid. The lyophilized purified sample was then chromatog. desalted with a recovery of >90%. Dynamic fast-atom bombardment selected ion monitoring \*\*\*mass\*\*\* \*\*\*spectrometry\*\*\* was carried out with and without a fused silica capillary column and a mobile phase of 1% trifluoroacetic acid + 5% glycerol in 50% aq. MeOH. The protonated mols. [D0]SP and [D8]SP were monitored at m/z 1368.7 and 1356.7, resp. Replicate injection (0.1 .mu.L) of 0.10-1.0 pmol [D0]SP analyzed in the absence of the microcolumn gave a limit of detection of 0.10 pmol. In the presence of the microcolumn there was a higher limit of detection of 0.10-0.25 pmol for [D0 + D8]SP. In all cases the deuterated \*\*\*internal\*\*\* std. coeluted with the peak of the endogenous sample having both SP-like immunoreactivity and a mass corresponding to the protonated mol. of SP.